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From: Schmidt, Mary
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increases the growth potential of new capillaries. These results suggest that angiogenesis involves an initiation event regulated by factors in serum and promotional event inhibited by serum factors.

#415 Monday, April 22, 1996, 8:00–12:00, Poster Section 1

Synergy between retinoic acid and interferon-α in inhibiting angiogenesis induced by head and neck squamous cell carcinoma. Lingen, MW, *Polverini PJ, Bouck NP. *Northwestern University Medical School, Chicago, IL. and *The University of Michigan School of Dentistry, Ann Arbor, MI.*

When used as single agents, retinoic acid (RA) and interferon-alpha (IFN) have shown great promise in preventing the development of new head and neck squamous cell carcinomas (HN SCC) in individuals at high risk, but the mechanisms by which these agents act are not fully understood. Using the *in vitro* capillary endothelial cell migration and the *in vivo* rat cornea assays, RA and IFN were found to inhibit HN SCC induced angiogenesis by different mechanisms. RA caused human HN SCC cell lines, but not lines from a fibrosarcoma, colon carcinoma, or breast carcinoma, to switch from an angiogenic to antiangiogenic phenotype by inducing the secretion of an inhibitor of angiogenesis. IFN treatment of these same HN SCC cells also influenced angiogenesis, causing a dramatic drop in their production of IL-8, which is the major inducer of angiogenesis secreted by these tumors. When used together, RA and IFN acted synergistically to decrease tumor cell angiogenic activity. In addition, the combination of drugs acted synergistically to inhibit the *in vitro* migration and proliferation of microvascular endothelial cells, allowing doses of each drug to be reduced by two logs without loss of activity. When rats were treated systemically with RA and IFN, the induction of corneal neovascularization *in vivo* by IL-8 was inhibited when 1/2 and 1/5 the minimal effective systemic doses of RA and IFN were used. These data suggest that the antiangiogenic activity of retinoids may play an important role in their ability to block the development of HN SCC and imply that their use in combination with IFN could be of significant benefit in the chemoprevention of this aggressive neoplasm.

#416 Monday, April 22, 1996, 8:00–12:00, Poster Section 1

Molecular modulation of vascular endothelial growth factor (VEGF) expression in glioma cells by a ribozyme. Ke, L.D., Chen, X.S., Steck, P.A., Yung, W.K.A., *University of Texas M.D. Anderson Cancer Center, Houston, TX, USA*

VEGF, a potent angiogenic growth factor, has been shown to be over expressed in human glioblastomas. It induces angiogenesis in a paracrine fashion since VEGF receptor is found only on endothelial cells. Nineteen potential GUC ribozyme cutting sites were identified in the human VEGF mRNA sequence. We have designed and constructed two ribozyme motifs (vRZmI, vRZmII) that cut the VEGF mRNA in exon I–III region that is common to all 3 VEGF variants observed in gliomas. In vitro study has demonstrated efficient digestion of a 309 bases VEGF RNA (vRZt) sequence in the predicted sites. VRZmI & II were cloned into pCEP4 vector for transfection studies. Western blot analysis showed decreased level of VEGF protein in both vRZmI and vRZmII transfected U251 cells. Single cell clones were isolated for further characterization. Initial quantitative PCR demonstrated lower level of VEGF mRNA in vRZmII transfected clones suggesting that vRZmII may be more efficient than vRZmI. These results suggest that ribozyme approach can be a potential molecular strategy to regulate the expression of VEGF in human glioma.

#417 Sunday, April 21, 1996, 1:00–5:00, Room 30

Elucidation of early events in tumor-induced angiogenesis. C. Tobias, J.A. Bryan, A. Filie, and S.W. McLeskey, *Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC 20007.*

Early events in neovascularization of tumors were studied by injection of FGF-4 transfected and parental MCF-7 cells into ovariectomized nude mice. Under these conditions, parental cells form static nodules which ultimately regress, while FGF-transfected cells form rapidly growing tumors. When examined up to 35 days after injection, tumors produced by parental and FGF-4 transfected cells contained proliferating tumor cells labeled with bromodeoxyuridine (BrdU). By Day 5, neovascularization was taking place in tumors produced by FGF-4 transfectants and by parental cells, as evidenced by PECAM immunohistochemistry. PECAM-positive neovessels were initially seen in the subcutaneous tissues immediately adjacent to tumor cells as well as intratumorally. By day 15, tumors formed by parental cell injection had mostly peripheral neovessels, which by day 20, formed a halo of ectatic blood vessels around the regressing tumor. Tumors formed from FGF-transfected cells continued to exhibit intratumoral microvessels. Tumor sections produced by either parental or FGF-transfected cells were scored for the amount of ectatic, peripheral blood vessels and for the amount of BrdU incorporation into tumor cells. Regardless of cell line injected, the score for ectatic peripheral blood vessels in a given tumor section was inversely correlated with the score for BrdU-labeled tumor cells, indicating that fast-growing tumors possessed intratumoral microvessels, while slow-growing had ectatic, peripheral vessels. No correlation was noted between the degree of inflammatory infiltration and the presence of either blood vessel phenotype. Thus, although both parental and FGF-transfected MCF-7 cells initially have the ability to stimulate neovascularization in ovariectomized nude mice, as the parental cell tumors regress, the blood vessel morphology changes.

#418 Tuesday, April 23, 1996, 10:40–10:55, Room 33

Characterization of the metastatic properties of FGF-1 transfected MCF-7 breast cancer cells. Zhang, L., Kharbanda, S., Kern, F.G. *Lombardi Cancer Center, Georgetown University Medical Center, Washington, D.C., 20007*

We have reported that FGF-1 and β-galactosidase overexpressing MCF-7 cells develop lung micrometastases in tumor-bearing nude mice but macroscopic foci are not observed. Tail vein injection studies ruled out the possibility that the appearance of cells in the lungs of tumor-bearing mice was due to an injection artifact. When cells were inoculated intravenously into the tail vein, numerous cells could be detected by X-gal staining when the mice were sacrificed two hours later but these cells were cleared within 24–96 hrs. When breast cancer cell lines established from the lungs of tumor-bearing mice were injected intravenously, they also failed to form macroscopic foci and are also eventually cleared. Primary tumors of FGF-1 overexpressing cells were allowed to develop for three weeks. When mice are sacrificed at this point, micrometastases are widely dispersed in the lungs. The primary tumors were resected from one-half of the tumor-bearing animals whereas tumors were allowed to continue to grow in the remaining half. After an additional four weeks, both groups of mice were sacrificed and the lungs were stained with X-gal. No metastases were found in any of the mice that had their primary tumor removed whereas the mice that continued to bear tumors had micrometastatic deposits. These results suggest that a primary tumor derived inhibitor of metastasis is not the cause of the failure of the FGF-1 overexpressing cells to develop into macroscopic foci and that the micrometastases detected in tumor bearing mice is the result of dissemination facilitated by FGF-1 induced neoangiogenesis. They also suggest that the subsequent clearance of FGF-1 overexpressing cells reflects the requirement for additional phenotypic alterations that will allow them to either extravasate or proliferate.

#419 Tuesday, April 23, 1996, 08:55–09:10, Room 33

Inhibition of endothelial cell capillary-like growth (tube formation) by an antibody specific for a new protein, angio-associated migratory cell protein. Beckner, M.E. and Liotta, L.A. *National Cancer Institute, NIH, Bethesda, MD 20892.*

AAMP, angio-associated migratory cell protein, is a newly identified protein with a potential heparin binding region. This region, as a peptide (P189), has a K_d = 14 pmol. Polyclonal antibodies to recombinant AAMP (rAAMP) have been affinity purified, tested for specificity, and used to characterize AAMP. Immunofluorescent staining shows that AAMP can be found in both the cytoplasm and in the immediate extracellular environment of cultured endothelial cells. AAMP's functional activity was studied in an assay for endothelial tube formation. This process can be elicited by plating HUVEC (human umbilical vein endothelial cells) overnight on Matrigel, a basement membrane substitute. Anti-rAAMP inhibits endothelial tube formation in a range of 64% to 3.9X at 1–10 μg/ml and 12% to 3.6X at 30 μg/ml, when tube formation is approximately 50–75% maximal for a cell concentration of 25,000/ml. Dilution curves have shown peaks of inhibition most often at 10 μg/ml of anti-rAAMP. Inhibition can be reversed with either cell concentrations that allow maximal tube formation (Matrigel dependent), higher levels of antibody, or heparin. P189 can also inhibit endothelial tube formation.

#420 Tuesday, April 23, 1996, 10:10–10:25, Room 33

Role of nitric oxide in tumor microcirculation. Fukumura, D., Yuan, F., and Jain, R.K. *Mass. General Hospital and Harvard Medical School, Boston, MA 02114*

Role of nitric oxide (NO) was determined through the direct intravital observations using NO synthase inhibitor and NO donor regionally and systemically (L-NAME 100μM superfusion, 10mg/kg injection; supermine NO 100μM superfusion, 10μmol/kg injection). Tumors (MCaIV: a murine mammary adenocarcinoma, LS174T: a human colon adenocarcinoma) were implanted in the dorsal skin chamber of C3H or SCID mice and observed under intravital fluorescence microscope. RBC velocity, vascular diameter, leukocyte-endothelial interactions and vascular permeability were measured. Maximum change in microvascular flow rate of regional NO inhibitor, donor, systemic NO inhibitor and donor group are −43%, +34%, −26% and −13%, respectively. Rolling and stable adhesion of leukocytes were significantly increased by NO inhibitor. Under control condition, both MCaIV and LS174T tumor vessels were leaky to macromolecules such as albumin. Systemic NO inhibition significantly attenuated tumor vascular permeability of MCaIV tumor but not of LS174T. These results suggest that endogenous NO increases tumor blood flow via vessel dilatation, decreases leukocyte-endothelial interactions and increases vascular permeability. The magnitude of response to NO is tumor dependent. Modulation of NO level is a potential strategy for improving tumor hemodynamics, radiation therapy and derivery of drugs to tumors.

#421 Monday, April 22, 1996, 1:00–5:00, Poster Section 2

Hydraulic conductivity of solid tumors: A novel in vivo measurement technique. Boucher, Y., Brekken, C., Netti, P.A., Baxter, L.T. and Jain, R.K. *Dept. of Radiation Oncology Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.*

We have developed a new technique to characterize and manipulate fluid transport parameters in tumor tissue that influence the systemic and intratumoral delivery of therapeutic agents. The hydraulic conductivity (K) of tissue is a quantitative estimate of the ease of fluid movement through that tissue by bulk flow. For estimating K *in vivo*